Epidermal Langerhans Cells Are Resistant to the Permeabilizing Effects of Extracellular ATP: *In Vitro* Evidence Supporting a Protective Role of Membrane ATPase

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Extracellular adenosine 5′-triphosphate (ATP_o) can induce pore formation in cell membranes, leading to cell permeabilization and eventual cell death. In this study, we examined the sensitivity of human epidermal Langerhans cells to ATP-induced permeabilization and tested the possibility that the Mg^{++}- or Ca^{++}-dependent plasma membrane ectonucleotidase (mATPase) on Langerhans cells provides protection against the cytotoxic effects of ATP_o. Membrane permeability was assessed by using the fluorescent tracer propidium iodide, which confers red nuclear fluorescence to permeabilized cells. Langerhans cells were identified within human epidermal cell suspensions with fluorescein isothiocyanate–conjugated MoAb against CD1a or human leukocyte antigen-DR (HLA-DR) antigens. Cultured human keratinocytes and J774 macrophages were both highly sensitive to permeabilization induced by incubation with ATP (0.5 to 20 mM at 37°C), whereas Langerhans cells were relatively resistant. The non-hydrolyzable ATP analog, adenosine 5′-(β,γ-imido) triphosphate, but not other nucleotides such as ADP, AMP, GTP, or UTP, was also able to induce permeabilization comparable to that of ATP, thereby suggesting that ATP hydrolysis is not required for this effect. ATP^{4-} is the moiety most likely responsible for permeabilization, because propidium iodide uptake occurred only when the pH of the medium was ≥ 7.4. Permeabilization induced by ATP was augmented by chelation of divalent cations with ethylenediaminetetraacetic acid and by the addition of lanthanum or cerium (0.01 to 1 mM). Finally, incubation with the adenosine analog, 5′-p-fluorosulfonylbenzoyl-adenosine (1 mM), inhibited mATPase staining of Langerhans cells in human epidermal sheets, but markedly augmented ATP-induced permeabilization of Langerhans cells. The results indicate that epidermal LC are resistant to the lytic effects of ATP_o and that mATPase is involved in such resistance. *J Invest Dermatol 100:282–287, 1993*

Langerhans cells (LC) are dendritic leukocytes that reside in stratified squamous epithelia, where they function as the principal antigen-presenting cells for T lymphocytes. LC possess a formalin-resistant adenosine triphosphatase (ATPase) that has been used extensively and reliably as a histochemical marker for these cells both *in situ* and in suspensions [1–5]. This ATPase is located on the plasma membrane with its active site facing the cell exterior, thus its designation as a membrane ATPase (mATPase) and an ectoenzyme [3].

mATPase on LC possesses a broad range of nucleotide-hydrolyzing activity that varies depending on the species. Mouse LC are more intensely stained by adenosine 5′-diphosphate (ADP) than by adenosine 5′-triphosphate (ATP) [5], whereas human LC display identical intensity of staining with ATP, ADP, or even inosine 5′-triphosphate [1]. By contrast, rat LC differ from their human and

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Abbreviations:
ADP: adenosine 5′-diphosphate
AMP: adenosine 5′-monophosphate
AMP-PNP: adenosine 5′-(β,γ-imido)triphosphate
ATP: adenosine 5′-triphosphate
ATPase: adenosine triphosphatase
ATP_o: extracellular ATP
cLC: cultured LC
CTL: cytotoxic T lymphocytes
DMEM: Dulbecco's minimum essential medium
DMSO: dimethyl sulfoxide
DNA: deoxyribonucleic acid
EC: epidermal cells
EGF: epidermal growth factor
FCS: fetal calf serum
FITC: fluorescein isothiocyanate
fLC: freshly isolated LC
FSBA: 5′-p(fluorosulfonyl)benzoyladenosine
GTP: guanosine 5′-triphosphate
HLA-DR: human leukocyte antigen-DR
LC: Langerhans cells
MoAb: monoclonal antibody
mATPase: membrane ATPase
PBS: phosphate-buffered saline
PI: propidium iodide
sRPMI: supplemented RPMI
UTP: uridine 5′-triphosphate
UVB: ultraviolet B

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mouse counterparts by their ability to stain with adenosine 5′-monophosphate (AMP) [5]. Activity of mATPase on LC requires divalent cations (e.g., Mg^{++} or Ca^{++}) and is insensitive to the membrane Na^+/K^+ ATPase inhibitor, ouabain [5].

Extracellular ATP (ATP_o) can alter several cell functions and cell membrane properties. It can depolarize plasma membranes, activate phospholipase C, and increase intracellular [Ca^{++}] [6–11]. In addition, ATP_o can increase cell membrane permeability, allowing molecules as large as fura-2 (831 D) to enter the cytoplasm, leading eventually to cell death [12–14]. ATP_o has also been suggested to serve as a mediator of cytotoxic T lymphocyte (CTL)-induced cytotoxicity, by causing cell lysis or DNA fragmentation [15–18]. Resistance of particular cell types to these permeabilizing effects of ATP_o has been attributed to the presence in these cells of an ecto-ATPase that can rapidly inactivate ATP_o [19,20].

In the present study, we examined the permeabilizing effects of ATP_o on epidermal LC and tested the hypothesis that mATPase on these cells provides protection against ATP_o-induced permeabilization.

MATERIALS AND METHODS

Chemicals ATP, ADP, AMP, uridine 5′-triphosphate (UTP), guanosine 5′-triphosphate (GTP), adenosine 5′-(β,γ-imido) triphosphate (AMP-PNP), 5′-p(fluorosulfonyl)benzoyladenosine (FSBA), propidium iodide (PI), cerium chloride heptahydrate (Ce^{+++}) and lanthanum chloride heptahydrate (La^{+++}) were purchased from Sigma Chemical Co. (St. Louis, MO).

Epidermal Cell (EC) Suspensions Skin was obtained from adult patients undergoing plastic surgery. Keratomized skin was cut into small pieces and then floated in 0.5% dispase (Neutrale protease grade II, Bohringer Mannheim, Mannheim, Germany) for 60 min at 37°C. Epidermis was separated from dermis and placed in 0.25% trypsin (Biochrom KG, Berlin, Germany) in phosphate-buffered saline (PBS) solution for 20 min at 37°C. Stratum corneum, hair, and other debris were filtered through sterile gauzes and Sera-Separa columns (Evergreen Scientific, Los Angeles, CA). Disaggregated EC were washed in Dulbecco's minimum essential medium (DMEM) augmented with 4.5 g/l D-glucose and 2.4 g/l sodium bicarbonate (Biochrom), and then resuspended in RPMI 1640 supplemented with heat-inactivated 10% fetal calf serum (FCS), 25 mM HEPES, 0.1 mM non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate (all Biochrom), 0.05 mM 2-mercaptoethanol (Merck, Daemstadt, Germany), and 1% penicillin-streptomycin (Biochrom) (supplemented RPMI [sRPMI]). EC viability was estimated by trypan blue exclusion to be 85–97%.

LC Enrichment EC suspensions were placed in 10-cm tissue culture dishes at 37°C with 5% CO_2, at a density of $40–60 \times 10^6$ cells/dish. After 60 min, the non-adherent fraction was collected and centrifuged; cells were then renumerated. EC suspensions were subjected to density centrifugation using a Ficoll-Paque gradient (1.077 g/ml) (Pharmacia, Uppsala, Sweden). Cells from the interface were harvested and washed in DMEM; interface EC routinely contained 5–10% LC.

EC Cultures Bulk EC suspensions were seeded in sRPMI in 10-cm tissue culture dishes (37°C; 5% CO_2) at a density of 40 to 60×10^6 cells/dish. After 48 to 72 h, the non-adherent fraction was collected and subjected to density gradient centrifugation, as described previously [21]. In some experiments, the non-adherent fraction was collected at 24 h and cultured in fresh medium for an additional 1–2 d. The viability of interface cells after culture ranged from 60–80%.

Immunofluorescent Staining of LC DMEM supplemented with 10 mM HEPES and 1% FCS was used to wash cells and dilute antibodies. EC were incubated with either fluorescein isothiocyanate (FITC)-conjugated anti-HLA-DR (0.5 $\mu g/10^6$ cells) (Clone L243; Becton Dickinson, Mountain View, CA) or anti-CD1a monoclonal antibody (MoAb) (OKT6, 1:20 dilution) (Ortho Diagnostic System, Raritan, NJ) for 30 min on ice, then washed, and finally resuspended in permeabilization assay medium.

Culture of J774 Macrophages J774 cells were obtained from American Type Culture Collection (Rockville, MD). Cells were cultured in 10-cm tissue culture dishes in DMEM supplemented with 2 mM L-glutamine and 10% FCS. For permeabilization assays, cells were detached by pipetting and then resuspended in permeabilization assay medium.

Culture of Human Keratinocytes Primary cultures were established from normal adult human skin procured following cosmetic surgery. Keratomized skin fragments were floated in 0.5% dispase for 1 h at 37°C. Epidermis was separated from dermis and then placed in 0.25% trypsin/0.02% EDTA solution for 20 min at 37°C. Disaggregated EC were filtered, washed, and then cultured according to Rheinwald and Green [27]. EC were seeded in 75-cm^2 culture flasks at 15 to $20 \times 10^3/cm^2$ on a feeder layer of mitomycin-treated (4 μg/ml for 2 h; Sigma) 3T3/J2 cells and then cultured in DMEM/Ham F12 medium (3:1) (Biochrom) containing mouse epidermal growth factor (EGF) (10 ng/ml; Sigma), cholera toxin (0.1 nM; Sigma), bovine insulin (5 μg/ml, Sigma), hydrocortisone (0.4 μg/ml; Calbiochem, San Diego, CA), transferrin (5 μg/ml; Sigma), adenine (18 mM, Sigma), triiodothyronine (2 nM; Sigma); 1% penicillin-streptomycin (Biochrom) and 10% FCS. At 70–80% confluence, keratinocytes were detached, using 0.05% trypsin/0.02% EDTA solution, and then subcultivated in defined serum-free medium consisting of MCDB 153 with 0.15 mM $CaCl_2$ (GIBCO, Uxbridge, England) supplemented with 10 ng/ml mouse EGF, 0.4 μg/ml hydrocortisone, 5 μg/ml bovine insulin, 70 μg/ml bovine pituitary extract (Collaborative Research, Cambridge, MA), and 1% penicillin-streptomycin. In permeabilization assays, keratinocytes were incubated in 0.1% EDTA solution at 4°C for 15 min, and then detached by pipetting.

Permeabilization Assay LC-enriched suspensions stained with FITC-conjugated anti-HLA-DR or anti-CD1a MoAb, J774 macrophages or cultured keratinocytes were suspended in DMEM containing 20 mM HEPES and augmented with different amounts of nucleotides (pH 7.8 ± 0.2), and then incubated for different time periods in a Dubnoff incubator at 37°C. After incubation, 25 μM PI was added and the cells were immediately analyzed under a Zeiss epifluorescent microscope. Only cells that displayed a bright red fluorescent nucleus were considered to be permeabilized. For each experimental condition, 90 to 350 LC, and greater than 300 keratinocytes or J774 cells were evaluated.

ATPase Staining of Epidermal Sheets Keratomized human skin specimens were treated with 0.5% dispase for 1 h at 37°C to separate epidermis from dermis. ATPase staining was performed on epidermal sheets as described [4]. Briefly, epidermal sheets were fixed in a cacodylate formaldehyde solution at 4°C, washed in Tris-mal buffer, and then stained with ATP/$Pb(NO_3)_2$ solution for 30 min at 37°C. After extensive washing, a reaction was revealed by the addition of 0.5% ammonium sulfide for 5 min at room temperature. Epidermal sheets were then washed and mounted in glycerin/PBS.

RESULTS

Freshly Isolated LC are Relatively Resistant to the Permeabilizing Effects of ATP_o ATP_o induces pores in plasma membranes that allow molecules of less than 900 D to enter cells [12]. In this study, PI was used as an indicator of ATP-induced permeabilization based on its molecular size (668 D) and its ability to intercalate into DNA, thus producing bright red nuclear fluorescence [23]. LC were identified by staining EC suspensions with FITC-conjugated MoAb directed against HLA-DR or CD1a. Permeabilized LC appeared under the fluorescent microscope as cells with red nuclei and green cell membranes.

A small percentage of freshly isolated LC (fLC) was stained by PI following exposure to increasing concentrations of ATP at 37°C for 30 min (Fig 1); maximal permeabilization (<25%) was achieved at

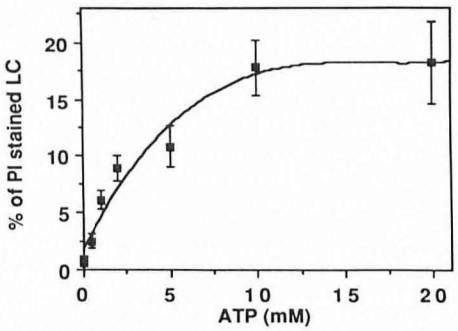

Figure 1. Only a small percentage of fLC are stained by PI following incubation with ATP. Freshly procured human EC suspension enriched for LC were stained with FITC-conjugated anti-HLA-DR MoAb, and then incubated with increasing concentrations of ATP (37°C, 30 min, pH 7.8 ± 0.2). Following the addition of PI (25 μM), cells were immediately examined under a fluorescent microscope. Permeabilized LC appeared as membrane green cells with red nuclei. Data were pooled from five experiments (means ± SD).

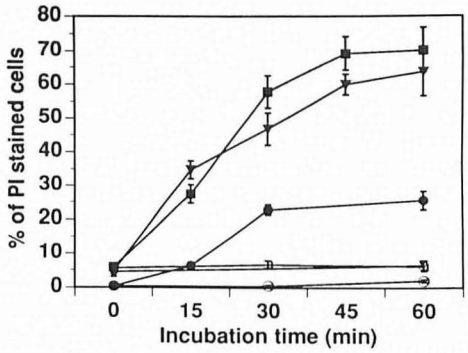

Figure 3. fLC are resistant to ATP_o-induced permeabilization when compared to cultured keratinocytes and J774 cells. Using the same conditions as in Fig 1, the effects of 10 mM ATP on fLC (●), cultured human keratinocytes (▼), and mouse J774 macrophages (■) were examined. *Open symbols* represent control samples incubated at 37°C without ATP. Data were pooled from three experiments (means ± SD).

a concentration of 10 mM ATP. Kinetic experiments indicated 30 min to be the optimal time to assess permeabilization (Fig 2). ATP-induced permeabilization was dependent on temperature, because staining with PI did not occur when cells were incubated at 0.5°C (Fig 2). To assess the reversibility of ATP-induced permeabilization of LC, EC suspensions were incubated with ATP at 37°C, washed twice with Hanks' balanced salt solution, and then stained with PI. Under these conditions, LC displayed PI staining similar to LC never exposed to ATP, suggesting that ATP-induced pores have been closed after removing ATP from the medium (data not shown).

We next compared fLC to cultured human keratinocytes and J774 mouse macrophages with respect to susceptibility to permeabilization by ATP_o. J774 cells were chosen for this comparison because they are known to be highly sensitive to ATP_o [11]. Both J774 cells and keratinocytes were considerably more sensitive than fLC to ATP-induced permeabilization (Fig 3).

Cultured LC are as Resistant to the Permeabilizing Effects of ATP_o as fLC Among the alterations that LC undergo during short-term culture [24] is a significant reduction [25] to complete disappearance of staining for mATPase [26]. On the other hand, other investigators have reported no differences in staining for mATPase between fLC and cultured LC (cLC) [27]. We have observed a 50–60% decrease in *total* ATPase activity expressed by membrane fractions obtained from purified human epidermal LC following culture, as measured by biochemical assay [28].

When incubated with ATP, cLC exhibited greater staining for PI than fLC (Fig 4). However, cLC also showed greater PI uptake than fLC, even in the absence of ATP, perhaps reflecting a reduced viability of cLC. When this higher background staining is accounted for by subtraction, the relative proportion of fLC and of cLC permeabilized by ATP is similar.

ATP and the Analog AMP-PNP, but Not Other Nucleotides, Possess Permeabilizing Properties Specificity of ATP-induced permeabilization was tested by examining the effects of other nucleotides. ADP, AMP, UTP, and GTP each failed to induce PI staining on LC (Fig 5*A*). By contrast, the non-hydrolyzable ATP analog, AMP-PNP, induced LC membrane permeabilization to a similar extent as that produced by ATP (Fig. 5*B*). This finding, which has also been reported for other cell types [12,29], suggests that the hydrolysis of ATP_o is not required to demonstrate its permeabilizing activity.

ATP^{4-} is the Active Species Responsible for Permeabilization ATP can be transformed into different moieties depending on the pH of and the presence or absence of divalent cations in the medium. Because the pK of the reaction $HATP^{3-} \rightleftarrows H^+ + ATP^{4-}$ is 6.95 [30], an increase in pH results in less amounts of protonated ATP, but more amounts of ATP^{4-}. Staining of LC with PI (in the presence of ATP) was minimal at acidic pH, but increased dramati-

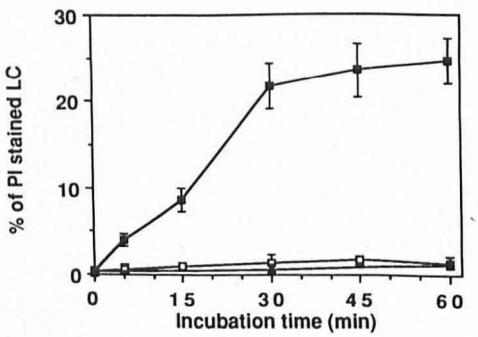

Figure 2. Time-course and temperature-dependence of ATP_o-induced permeabilization. Using the same conditions as in Fig 1, the effect of 10 mM ATP at two different temperatures, 37°C (■) and 0.5°C (□), were compared at different time periods of incubation. Control samples were incubated without ATP at 37°C (▲). Data were pooled from four experiments (means ± SD).

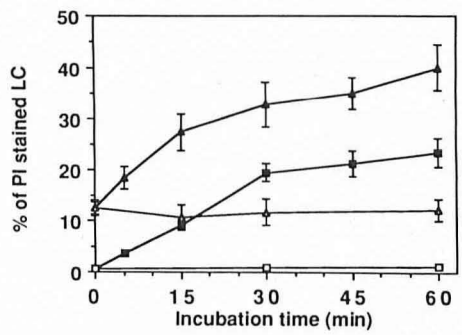

Figure 4. fLC and cultured LC (cLC) are similarly sensitive to ATP_o-induced permeabilization. Although cLC (24–48 h) (▲) exhibited higher PI uptakes compared to fLC (■), their background PI uptake (cLC [△] versus fLC [□], incubation at 37°C without ATP) was also higher. When this background staining is accounted for by subtraction, the percent of both fLC and cLC permeabilized by ATP is similar. Results were pooled from three experiments (means ± SD).

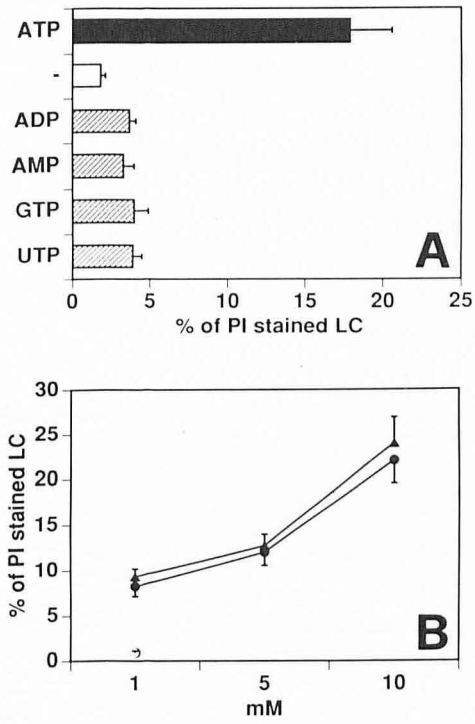

Figure 5. ATP and the non-hydrolyzable ATP analog AMP-PNP (but not other nucleotides) can permeabilize fLC. *A*, ATP, but not ADP, AMP, GTP, and UTP (each at 10 mM), induced PI uptake in fLC (37°C, 30 min). *B*, AMP-PNP (10 mM at 37°C for 30 min) induced PI uptake to the same degree as ATP: (●) ATP, (▲) AMP-PNP, (○) control sample without added nucleotides. Results were pooled from two experiments (means ± SD).

cally when pH rose beyond 7 (Fig 6). These results suggested that ATP^{4-} is the moiety responsible for permeabilization of LC, as has been shown for macrophages [12], mast cells [13], and fibroblasts [14].

Because divalent cations can bind to ATP and thus reduce the concentration of ATP^{4-}, we examined the effect of their removal from media. The addition of the cation chelator, EDTA, markedly increased (up to 85% after 60 min) the percentage of permeabilized fLC (Fig 7). By contrast, incubation with EDTA in the presence of equimolar concentrations of Mg^{++} (or Ca^{++}, data not shown) partially blocked this increase.

Inactivation of Membrane ATPase Markedly Increases the Sensitivity of fLC to ATP_o-Induced Permeabilization To determine whether mATPase can protect LC from the permeabiliz-

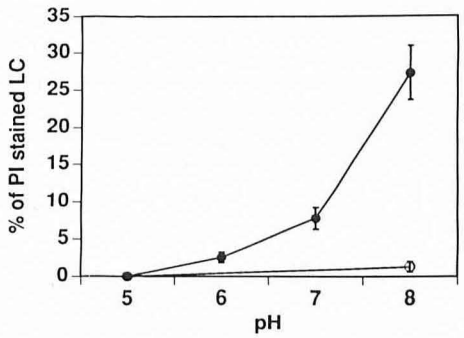

Figure 6. pH dependence of ATP_o-induced permeabilization on fLC. ATP-induced PI uptake by fLC was seen only when pH was greater than 7. (●) 10 mM ATP at 37°C for 30 min; (○) No ATP at 37°C for 30 min. Results were pooled from three experiments (means ± SD).

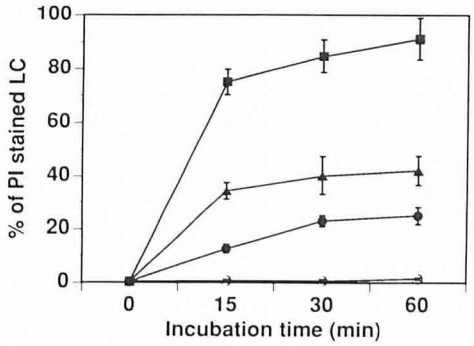

Figure 7. Chelation by EDTA of divalent cations increases ATP_o-induced permeabilization of fLC. Incubation with 10 mM EDTA augmented PI uptake in fLC, whereas incubation with 10 mM EDTA in the presence of $MgSO_4$ (10 mM) partially prevented this augmentation. (●) 10 mM ATP, 37°C; (○) 10 mM ATP, 0.5°C; (■) 10 mM ATP plus 10 mM EDTA, 37°C; (▲) 10 mM ATP plus 10 mM EDTA and 10 mM $MgSO_4$, 37°C. Data were pooled from three experiments (means ± SD).

ing properties of ATP_o, we examined the effects of an adenosine analog, FSBA, known to inhibit Na^+/K^+ membrane ATPase by covalently binding to the ATP binding site of the enzyme [31]. FSBA (1 mM) inhibited mATPase staining of LC in epidermal sheets (Fig 8). In addition, pre-incubation of EC with FSBA (30 min on ice), followed by incubation with ATP (30 min, 37°C), resulted in a marked increase in the percentage of LC stained by PI

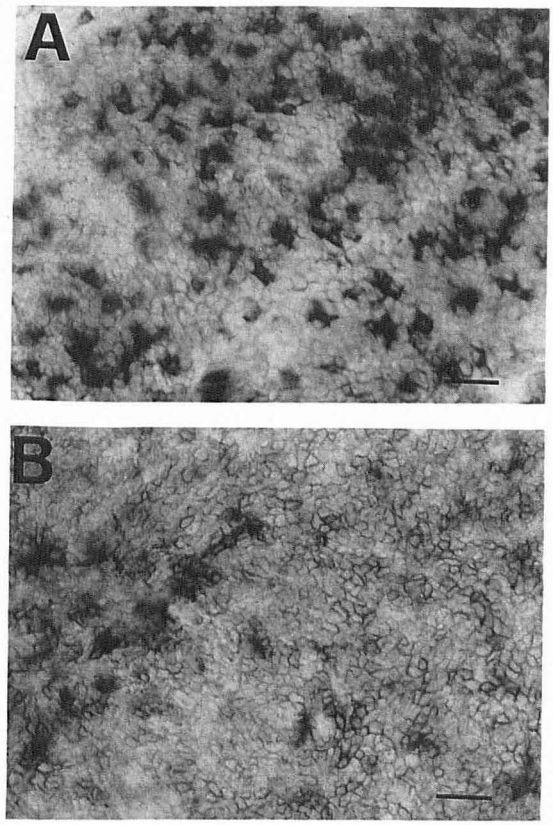

Figure 8. FSBA inhibits ATPase staining of LC in human epidermal sheets. Keratomized human skin specimens were treated with 0.5% dispase (1 h, 37°C) to separate epidermis from dermis. Epidermal sheets were then subjected to ATPase staining as described in *Materials and Methods*. *A*, Control epidermis, showing ATPase positive dendritic cells. *B*, Epidermis pre-treated with 1 mM FSBA. *Bar*, 50 μm.

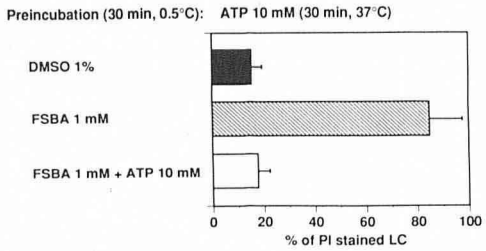

Figure 9. Pre-incubation with FSBA promotes ATP_o-induced premeabilization of fLC. fLC were pre-incubated (30 min, 0.5°C) with 1 mM FSBA *(striped bar)*, 1% dimethyl sulfoxide (DMSO) *(solid bar)*, or 1 mM FSBA plus 10 mM ATP *(open bar)*. 10 mM ATP was then added and incubation allowed to proceed for an additional 30 min at 37°C. Cells pre-incubated with FSBA alone displayed augmented PI uptake, whereas cells pre-incubated with DMSO or FSBA plus ATP showed limited PI uptake. Data were pooled from three experiments (means ± SD).

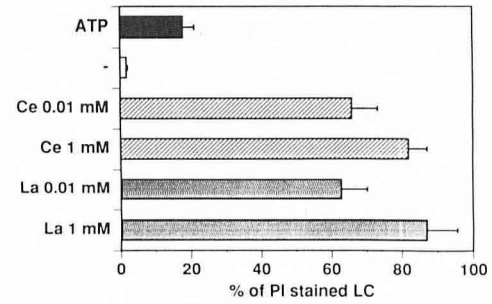

Figure 10. Ce^{3+} or La^{3+} enhance ATP_o-induced permeabilization of fLC. fLC were incubated (30 min, 37°C) with 10 mM ATP plus Ce^{3+} or La^{3+} (0.01 or 1 mM). At either concentration, Ce^{3+} or La^{3+} increased ATP-induced permeabilization of fLC. A control sample *(open bar)* was incubated (30 min, 37°C) without ATP. Data were pooled from two experiments (means ± SD).

(Fig 9). By contrast, pre-incubation with the solvent DMSO alone or FSBA plus ATP failed to increase permeabilization.

We next evaluated the effects of lanthanid ions, which compete with divalent cations to block mATPase staining of epidermal LC [32]. Incubation of EC with La^{+++} or Ce^{+++}, in the presence of ATP, resulted in a marked augmentation of the percentage of LC stained by PI (Fig 10). On the other hand, incubation with La^{+++} or Ce^{+++} did not induce LC permeabilization in the absence of ATP, ruling out a possible direct toxic effects of these ions on LC (data not shown).

DISCUSSION

A Mg^{++}-/Ca^{++}-dependent membrane ecto-ATPase with broad nucleotide hydrolyzing activity is present on epidermal LC and on many different cells, including endothelial cells [6], polymorphonuclear leukocytes [33], T lymphocytes [19], macrophages [34], hepatocytes [35,36], and pancreatic cells [37]. It is insensitive to known inhibitors of ion-translocating ATPases, such as ouabain, vanadate, oligomycin, and N-ethylmaleimide [5,37–39]. Because of its ability to hydrolyse ATP, mATPase can terminate several effects of ATP_o, including its cytotoxic (permeabilizing or lytic) activity. In concert with 5′-nucleotidase, ecto-ATPase can also produce free adenosine, which then becomes available for ligation of adenosine receptors and/or use by cells [6,7].

In this study, we observed LC, whether freshly isolated or cultured, to be permeabilized to only a small degree by ATP. By contrast, both cultured human keratinocytes and J774 mouse macrophages were permeabilized to a great degree by ATP_o. Permeabilization was dependent on the dose of ATP, length of time of exposure, and incubation temperature. Permeabilization was almost exclusively specific for ATP, because it was not observed when cells were incubated with other nucleotides, the exception being the non-hydrolyzable ATP-analog, AMP-PNP. ATP^{4-} is the most likely moiety responsible for permeabilization because PI staining occurred only when the pH of the solution was higher than 7 and was augmented by removing divalent cations from media. The markedly increased ATP-induced permeabilization following the removal of Mg^{++} or Ca^{++} is consistent with the strict divalent cation-dependence of mATPase activity. That mATPase on LC may be responsible for the relative resistance of LC to ATP-induced permeabilization is suggested by the marked augmentation of ATP-induced permeabilization of LC produced by the addition of either FSBA or lanthanids, both of which are established inhibitors of mATPase [19,20,31,32]. However, the presence of membrane ecto-ATPase alone may not be the only protective mechanism against ATP_o. CTL that are resistant to ATP-induced permeabilization express high ecto-ATPase activity [19], yet these cells retain their resistance to ATP-induced permeabilization even when incubated with EDTA at concentrations that inhibit mATPase activity [15].

The mechanism responsible for the ATP_o-induced permeabilization is not fully understood. Recent work in J774 macrophages has suggested connexin-43, a constitutive component of gap junction channels, as the mediator of the permeabilizing activity of ATP_o [40]. Relevant to this issue is the finding that the ATP_o-resistant J774 variant cell line, ATPR, does not express connexin-43 mRNA and protein [40]. It is thus possible that the resistance to LC to permeabilization by ATP_o is due to the absence of this protein. Connexin-43 has been identified in plasma membranes of rat and human keratinocytes [41], but has not been studied in LC.

ATP is a ubiquitous molecule found within all cells, and may thus be released following cell membrane damage [6]. In addition, ATP is secreted externally in a regulated manner by platelets, sensory neurons, adrenal chromaffin cells, and possibly CTL [6,7,18,19]. The results of our *in vitro* study led us to posit that mATPase may protect epidermal LC *in vivo* from permeabilization by ATP_o. ATP may be released in epidermis by sensory neurons, by platelets extravasated in the early phase of skin wounding [42], by infiltrating CTL during the course of inflammatory skin diseases [43,44], and by keratinocytes damaged by a variety of insults including ultraviolet B (UVB) radiation. UVB radiation produces morphologic and functional alterations in LC and alters their antigen-presenting ability [45,46]. In this respect, it is especially relevant to note that mATPase activity has been the phenotypic marker most consistently reported to be reduced on LC following UVB radiation [46,47]. In this scenario, UVB-irradiated LC, lacking mATPase activity, may become markedly susceptible to the adverse effects of ATP_o.

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